Appendix 5F

Tandem Mass Spectrometry Method for the Analysis of Amino Acids, Succinylacetone, and Acylcarnitines

Tracking Number CN 009

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Table of Contents

I. Title

Tandem Mass Spectrometry Method for the Analysis of Amino Acids, Acylcarnitines, and Succinylacetone.

II. Principle

Amino acids (AA), acylcarnitines (AC), and succinylacetone (SUAC or SA) are biochemical markers for the detection of inherited metabolic disorders, classified as amino acid, fatty acid oxidation and organic acid disorders, using tandem mass spectrometry (MSMS). Amino acids, succinylacetone, and acylcarnitines are extracted with the Extraction Solution (Methanol and H_2O) containing their internal standards from a 3.2 mm filter paper disk punched from a dried newborn blood spot. Hydrazine monohydrate solution is added to the Extraction Solution to allow the formation of a SUAC-hydrazine derivative to enable analysis for SUAC. 20 µL of the sample along with the Flow Solvent are then pumped into the ionization source to generate ions of the analyte(s).

The ionized sample is drawn into the mass analyzer for analysis. The first mass analyzer MS1 separates the parent ions based on their mass-to-charge ratios. Soft fragmentation is achieved with argon gas in the collision cell. The product ions formed in the collision cell are then propelled into the second MS2 analyzer, which allows daughter ions of a particular mass to pass on to the detector. Data are acquired in the Multiple Reaction Monitoring (MRM) mode. During this acquisition, a collisionally induced product of each analyte is measured for a set time period. Data acquisition and processing is performed by the software package included with the system.

The counts of the product ions are recorded by the detector for each analyte. The software algorithm compares the signal intensity of the analyte and the corresponding internal standard to determine the concentration of the analyte in the sample.

III. Specimen Collection and Type

The specimen is a dried blood spot approximately 1.5 cm in diameter collected on filter paper from a newborn heel stick puncture.

IV. Equipment and Supplies

A. Equipment

1. Supplied by Perkin Elmer

a. MS/MS analytical system, consisting of

1) Peak Scientific Nitrogen Generator
2) Edwards vacuum pump
3) Waters 1525 HPLC pump
4) Waters 2777C Autosampler
5) Waters Quattro micro tandem mass spectrometer
6) IBM PC to run tandem mass spectrometers
7) Server
8) HP printer
9) MassLynx and NeoLynx software
10) Branson Sonicator
11) POWERVAR Security Plus UPS

b. Wallac DBS Puncher with PC and barcode reader

c. Shaker TriNest Shaker-Incubator

d. Thermolyne Speci-Mix rocker

2. Supplied by NAPS Laboratories Fume exhaust hood

B. Supplies

1. Supplied by Perkin Elmer

a. PEEK tubing, 0.007" diameter, yellow

b. PEEK tubing, 0.005" diameter, red

c. PEEK tubing cutters

d. Autosampler syringe unit

e. In-line filter frits

f. 3.2 mm punch head

g. Solution troughs

h. Single channel pipettes, 1000 and 300 (replacement provided by NAPS Lab)

i. Multi-channel pipettes, 50 – 300 µL, Finnpipette from Fisher brand, (replacement provided by NAPS Lab)

j. Pipette tips, blue and yellow

2. Supplied by NAPS Laboratories

a. Volumetric pipettes

b. Kimwipes

V. Reagents

A. Supplied by PerkinElmer

1. NeoBase Non-derivatized MSMS Kit, contains enough reagents for 960 assays, store refrigerated, ship in 3 separate boxes

a. First box (MS/MS kit) contains:

(**NOTE:** If kit is not open for testing, leave the box intact as received, keep all the components in the box and store it at 2°C to 8°C. Do not mix SQC, Internal Standards, or barcodes between different kit lot numbers.)

1) Pre-barcoded dried blood spot controls (SQC), low and high levels, 3 bags, 1card/bag. Store these control bags at -10°C to -20°C when the kit is opened for testing.

2) Stable-isotope Amino Acids Internal Standards, stores at 2°C to 8°C, use until expiration date. .

3) Stable-isotope Acylcarnitine Internal Standards, store at 2°C to 8°C, use until expiration date.

4) V-bottomed 96 well NUNC plates, polypropylene, 10 plates.

5) Clear truncated V-bottom 96 well plates, polystyrene, 10 plates.

6) Barcode labels, 10 in triplicates.

7) Aluminum foil and adhesive covers, 10 sheets each.

b. Second box contains (If not in use, stores at 2°C to 8°C):

1) Flow solvent, Methanol and H_2O, 2 bottles, store away from light and heat.

2) Extraction solution, Methanol and H_2O, 1 bottle, stores away from light and heat.

c. Third box contains NeoBase Succinylacetone assay solution, hydrazine, stores at 2° to 8°C.

2. Argon gas cylinder, ultra high purity (UHP), 99.99%.

B. Supplied by GDL

1. Blood Spot Tray Control (CT), stores frozen at –10°C to –20 °C

2. Special Proficiency Control (CP) (same as CT), stores frozen at –10°C to –20 °C

C. Supplied by NAPS Laboratories

1. Methanol, HPLC grade

2. Distilled water or equivalent to NCCLS Type I water.

VI. Calibration and Quality Controls

A. Calibration

A set of calibrators at known concentration for each analyte is not run for this assay. Quantitation is relative to the signal of its internal standard.

B. Quality Controls

1. System Controls
The system controls (SQC) are dried human blood spots on filter paper. The two levels, low and high, are spiked with amino acids, acylcarnitines, and succinylacetone are run in duplicate, at the beginning and end of each tray, i.e., wells A4 and A5 at the beginning and wells H11 and H12 at the end of a full tray. These controls are part of the NeoBase Non-derivatized MSMS Kit supplied by PerkinElmer. System controls should not be punched more than 4 times per blood spot.

2. Tray Control
The tray control (CT) is dried human blood spots on filter paper. The analyte concentrations for the control are close to the cutoff. This tray control is prepared at GDLB and supplied to the NAPS laboratories as part of the GDLB quality control program. Tray controls are run in wells A6 and H10 of a full tray or in the position immediately after the last newborn specimen on the tray. Tray controls, should not be punched more than 4 times per blood spot.

3. Special Proficiency Control
Special Proficiency Control (CP) is identical to Tray Control except for its name and its location on the tray. CP is run in position E1.

VII. Procedures

A. Barcoding

Refer to the Newborn Screening Accession and Reporting at the NAPS Lab Protocol.

B. Warnings and Precautions

1. Only trained personnel should use this kit.

2. This kit contains components manufactured from human blood. The source materials have been tested by immunoassay for hepatitis B surface antigen, anti hepatitis C and anti-HIV 1 and 2 antibodies, and found to be negative. Nevertheless, all recommended precautions for the handling of blood derivatives should be observed. Please refer to the US Biomedical Laboratories and any other local or national regulation.

3. Handle all patient specimens as potentially infectious.

4. Follow local regulations for handling and disposal of hazardous waste.

5. Use fume hoods when handling reagents as specified.

C. Preparation of Reagents

Use the hood when handling Extraction Solution and SUAC assay solution.

1. Reconstitute the vials of dried Amino Acids Internal Standards (AA-IS) and Acylcarnitines Internal Standards (AC-IS):

a. Check the AA-IS and AC-IS vials to make sure the glass is not cracked or damaged, and caps are on tightly. If needed, tap the cap gently on the bench top to help removing the cap.

b. Use a 1.0mL volumetric pipette or a calibrated 1000µL single-channel-pipette and add 1.0mL of the Extraction Solution to each of the two IS vials. Avoid cross contamination between the vials.

c. Replace the caps and wrap them securely with parafilm.

d. Place them on the Thermolyne Speci-Mix rocker for at least to 2 hours for a thorough mix. Rotate the vials 180º after about one hour or ¼ turn every half an hour.

e. Check to make sure that all particles are dissolved and the IS solution is clear.

f. Label the vial with the reconstitution date, expiration date, and preparer's initial.

g. Store the vials at 2 - 8 °C (refrigerated) when not in use. This solution is stable for 30 days when kept in the sealed original vial at 2 - 8°C.

NOTE: Reconstitute the internal standard vials one day prior to their use.

2. Prepare daily working solution by diluting the AA-IS, AC-IS, and NeoBase Succinylacetone (SUAC) Assay Solution. Dilution factor for AA-IS and AC-IS is 1:110 and for NeoBase SUAC Assay Solution is 1:40 with the Extraction Solution. The volume for daily working solution for one full plate is 11mL.

a. Mix the AA-IS, AC-IS, and SUAC vials on the rocker for at least 10 minutes.

b. Use volumetric pipets, i.e., use a 10mL and a 1mL to add 11mL of Extraction Solution into a glass reagent bottle with a stopper.

c. Remove 475µL of Extraction Solution. Add 100µL of AA-IS, 100µL of AC-IS, and 275µL of NeoBase SUAC Assay Solution. As a precaution, work under a fume hood for this step.

d. Recap the flask with its stopper. Swirl gently to mix. Use as soon as possible to minimize evaporation. When wrap tightly, stored at 2°C to 8°C (refrigerated) and protected from light, the solution is stable for 24 hours.

e. To prepare for more than 1 plate, multiply 11mL, 100µL, and 275µL by the number of plates needed to get the volume to remove and volume to add. Use increments of ½ plate for partial plates. Refer to the table on the next page.

Prepare the Daily Working Solution for ½ or More Plates

No. of Plates	Final Volume of Working Solution in mL	Total volume Remove in µL	Volume in µL		
			AA-IS	AC-IS	SUAC
½	5.5	237.5	50	50	137.5
1	11	475	100	100	275
1 ½	16.5	712.5	150	150	412.5
2	22	950	200	200	550
2 ½	27.5	1187.5	250	250	687.5
3	33	1425	300	300	825
3 ½	38.5	1662.5	350	350	962.5
4	44	1900	400	400	1100
4 ½	49.5	2137.5	450	450	1237.5
5	55	2375	500	500	1375
5 ½	60.5	2612.5	550	550	1512.5
6	66	2850	600	600	1650
6 ½	71.5	3087.5	650	650	1787.5
7	77	3325	700	700	1925
7 ½	82.5	3562.5	750	750	2062.5
8	88	3800	800	800	2200

D. Punching

1. Assemble the accessed patient specimens for the day's run.

2. Take the required number of system controls and tray controls out of the freezer.

3. Put barcodes on a clear truncated V-bottom, a NUNC V-bottom plate and on the plate map using the set of triplicate barcodes.

4. Turn on the PC, press CTRL, ALT, Delete, enter username and password.

5. Turn on the DBS Puncher and select **Slave**.

6. Select Puncher Workstation icon on the PC.

7. Click **Load** at the top of the screen.

8. Scan the barcode on the clear truncated V-bottom plate, select **AAAC** for **Test:** and click **OK** to accept.

9. Load the plate onto the DBS puncher in the correct orientation: well A1 at the upper right corner and click **OK**.

10. Click **Specimen** at the top of the screen.

11. Click **Patient** to begin punching patient samples.

12. Scan the barcode of each patient blood spot specimen and punch (using 3.2 mm diameter punch head) into the corresponding well of the plate. Follow the plate map to complete the punching of the tray.

13. Once punching is completed for a full tray, a message box appears on the PC screen asking if you want to load the next tray, click **No**.

14. Click **Control** or follow step # 20 bellow to begin punching the QC samples.

15. Scan and punch the controls (SQC and CT; CP as needed) according to the plate map. After controls have been punched, a message box appears asking if you want to load the next tray, click **Yes** if applicable, otherwise, click **No.**

16. To unload the tray, a prompt appears: All wells for the plate barcode xxxxxxxxxxxxx have been punched. Do you want to unload this plate? Click **Yes**. Remove the plate from puncher and press **OK** on the puncher.

17. Select the MS/MS instrument (for laboratories with more than one MS/MS system) from the drop down menu on the Select Instrument screen. Click **OK**.

18. Select the autosampler sampling position. A prompt appears to select the "slot for plate xxxxxxxxxxxxx". Double click on the slot number to select that location. Click **OK**.

19. Scan another plate to continue or select **Cancel** to exit the punching application. For a partial tray, once the last patient specimen is punched, select **Cancel** from the Patient Barcode Entry screen.

20. Right click on the plate map and select **Mark as complete plate** xxxxxxxxxxxxx (plate barcode). Message box appears stating that the controls for that tray need to be punched and asks if you want to punch them now, click **Yes**.

21. Repeat steps 15 – 18 and select **Cancel** to exit.

22. Turn off the puncher and PC when done.

NOTES:

a. During punching, press **Check Plate** on the puncher to look for any missing blood spots. Press **Resume** to continue.

b. Do not punch more than 4 times from a dried blood spot for the SQC and TQC.

c. If an error occurs during punching, instrument or operator error, and a tray needs to be repunched, do the following or call Proxy.

1. Log on to the Supervisor's PC.

2. Select Result Viewer (MS/MS).

3. Select the At Worklist icon on the left side of the screen.

4. Select and open the tray to be repunched.

5. Prevent the tray.

6. Print the Repeat List. If the specimens do not appear on the Repeat List, call Proxy.

E. Extraction and Derivatization of Succinylacetone

1. Have sufficient supplies and reagents ready, i.e. pipette tips, adhesive covers, solution troughs, aluminum covers, and daily working solution for the day's run.

2. Turn on TriNest incubator/shaker unit at least 10 minutes before use. Press preheat button to start the preheating. Red LED will blink when target temperature is reached. Make sure the temperature is set at 45°C. Check temperature, time and rpm on the display. The rpm is set at 700 rpm and it is not necessary to check daily if no adjustment was done.

3. Use multichannel pipette and recommend doing reverse pipetting technique (initially pick up 100 µL twice, dispense 100 µL, pick up 100 µL, dispense 100 µL, etc.), add 100 µL of the daily working solution to each well containing a filter paper disc. The same pipette tips may be used for the entire tray. Avoid touching the sample discs at the bottom of the wells.

NOTE: The volume of working solution added is critical.

4. Seal the tray with an adhesive plastic cover. Check to make sure the seal over the wells is smooth and tight so evaporation can be minimized. Record the starting temperature reading on the log sheet.

5. Pull out incubator tray holder from position 1, 2, or 3.

6. Place the sealed clear sample tray on the holder properly.

7. Return the incubator tray holder containing the sample tray back into its original slot in the incubator/shaker.

8. Press the start button (where four corners lights are flashing) on the incubator/shaker to start the incubation.

9. The incubator will shake for 45 minutes at 45 ±1°C at 700 rpm setting. When the extraction incubation is finished, the instrument will beep, stop shaking, and terminate heating. A small blue light will flash at the finished tray position display.

10. Record the ending temperature reading on the log sheet right before the time is up. The incubator/shaker will shut off the heat when it stops unless there are other trays present.

11. Remove the finished tray from the incubator/shaker. Place the tray on a safe place until step 15 can be performed which should be immediately following steps 12 and 13 below.

NOTE: Check to make sure that all blood spots settled at the bottom of the wells. If a blood spot is not in the solution at the end of the incubation, elution may not occur properly. That sample or tray needs to be repeated depends on the location of the blood spot.

12. Replace the empty tray holder back into the incubator.

13. Press the start button to resume the incubation for the remained tray(s) and repeat steps 10-12. Turn off the instrument when finished.

NOTE: For any newly loaded tray, the incubation timer will start from the beginning at that location. For any tray that has not been removed, the timer will resume from when it was stopped.

14. To change a program for the incubator/shaker (also refer to instrument manual booklet):

a. The current parameters for Program 01 will be displayed when instrument turns on.

b. To see another program, turn the **Selection Dial** until the desired program number is displayed.

c. After selecting the program number, press the **Right Arrow** or **Left Arrow** to select the right program for test. Here you can name your program.

d. When you press the Right arrow after the sixth position, the cursor will move to the incubation temperature value.

e. To set the Temperature at 45ºC, Incubation Time at 45 minutes, and Shake Speed at 700 rpm (acceptable range is 650-750 rpm), Interval Time 0 minutes for the parameters, use the **Selection Dial** and the **Arrow buttons**.

f. Press the Right Arrow after the above settings are selected. The editing mode will close and parameters will be saved.

15. Place the tray from step 11 on a flat surface and carefully remove the adhesive plastic cover to avoid splashing. Use the Apricot Pipettor to transfer 75 µL from each well of the clear tray to the corresponding wells on the NUNC V-bottom tray that was pre-barcoded with the same barcode ID.

NOTE: It is best to transfer immediately after removing from the incubator/shaker to avoid condensation. It you cannot transfer immediately, it is best to keep the microtiter plate in the incubator/shaker for a short time.

a. Turn on the Apricot computer before incubation time is up, enter user name and password. Turn on the Apricot pipettor.

b. Open the Apricot icon on the desktop and select **Yes** to initialize the Apricot pipettor.

c. Oil the Apricot head O-rings by placing the head in the intended lubricating box, then attach the head to a set of pipette tips using the tip loader. Position the head with pipette tips onto the Apricot. Remove the pipette tips from the tip head using the tip loader at the end of each day and replace the tips every day.

d. Go to **File → Open → NeoBase transfer → Run → Verification Table.**

e. Place the eluted clear tray on Station 1.

f. Place the NUNC tray with the same barcode ID on Station 2.

g. Place a clean wash Reservoir with DI water, ~ ½ to ¾ full, on Station 4.

h. Select **Check Head**, **Check Plates**, **Check Spacers**, then click **OK** to start the pipetting process. Observe the liquid level in the tips at aspirate and dispense steps for proper pipetting by the instrument. Do trouble shooting if the liquid level of one or more tips is low.

i. Remove the NUNC plate from the Apricot and cover the plate with aluminum foil to minimize evaporation. Smooth out the aluminum foil on the tray with a clean kimwipe. Write the tray's ID (last four digits), date, and current time on the foil and off to the sides or edges of the tray. Avoid writing on top of the wells.

j. Allow at least 2 hours from transfer to sampling to allow complete derivatization of extracted succinylacetone. During the 2 hours, it is best to keep the plate in the selected location in the autosampler stack drawers.

F. Import Worklist

1. Select **File → Open → Import Worksheet** on the MassLynx sample list screen, Select **TAB Delimited** (*.TDL, *.TAB, *.TXT) From the Files type drop down menu.

2. Select the correct worklist ID from the list based on the tray barcode (e.g. **01262114900001.TDL**). Once selected, the ID will appear in the File Name box. In the File Name box, use the mouse to right click to highlight the ID but leave out **.TDL**. Copy the file name by pressing Ctrl + C. Click **Open** to bring the tray into the sample list screen.

3. Select **File → Save As** and paste the ID in the File Name box where the cursor is flashing by pressing Ctrl + V and click **Save** under the appropriate project.

4. Repeat steps 1-4 for each tray to be run.

G. Set-up for Analysis

1. Leave the drawer stack on the Sample Manger the way the MassLynx left it after shutdown. If the drawer was close, leave it closed. If it was open, then leave it opened.

2. Turn on the PC or Restart the PC so the system can reset itself.

3. Enter username and password.

4. Click on the **MassLynx** icon to open MassLynx.

5. Create a new project on a weekly basis.

a. Under **File**, select **Project Wizard**. Click **Yes** when prompt.

b. Enter in the **Project Name**: New projects will be created weekly. Each project will be named following the same format:

LAB&SYS#_WEEK NUMBER&YEAR

(e.g.**GD1MM1_2712** for GDL, Sys1, week 27 and year 2012)

c. Verify **Location** reads **E:\Projects\2012Projects\,** click **Next.**

d. Select **Create Using Existing Project as Template** (the last option). Be sure **a Copy sample list is** checked.

e. Click **Browse** and select **C:System** from **Directories**.

f. Click on **MassLynx** from the top Directories. Then select **NeoBase AAAC_ND_MASTER.PRO** from the left column. Click **OK**.

g. Existing project should read: **C:\MassLynx\NeoBase_AAAC_ND_MASTER.PRO**

h. Click **Finish**.

NOTE: A new project should be created **before** the daily run at the beginning of each week on each system.

6. Verify the following:

MS Tune File, **MS Method**, and **Inlet file** show **NeoBase_AAAC_ND, Well** is **1:1** (for 1st well on tray in position 1), **Inject volume** is **30** µL, **Sample** is **Analyte**, **Process** is **NeoLynx**, and **Parameter file** is **xxxxxx-Param.ntf** (**xxxxxx =** current kit lot #).

NOTE: Confirm that the MS Tune File's temperatures are as follow:

Source Temp = 120 ºC and Desolvation Temp = 350 ºC

Confirm that the MS Method MRMs are in the following order:

MRM of 25 mass pairs	Time 0.00 to 1.50, ES+
MRM of 21 mass pairs	Time 0.00 to 1.50, ES+
MRM of 25 mass pairs	Time 0.00 to 1.50, ES+

7. Verify waste containers are less than ¾ full.

8. Verify volume of Flow Solvent is sufficient for the run. The brown bottle is sufficient for light protection and is not necessary to foil wrap the bottle. The cap fittings are not sufficiently tight for the brown bottle. Tightly wrap Parafilm around the cap fittings on the bottle to minimize evaporation.

9. Verify volume in the Wash Bottle is sufficient for the run. Fill the Wash Solvent bottle with Flow Solvent as needed (at least 150 mL).

NOTE: Also verify that there is a Wash Solvent bottle connected to Wash Station 2.

10. Check the Teflon and PEEK tubings for cracks, leaks, clogs and bubbles.

11. Change the in-line filter frits <u>every other day or every two trays</u>. Flush Filter Frits, Teflon tubing, and sample injection port with methanol daily. Take care not over tighten the in-line filters assembly.

12. Check the nitrogen generator outlet pressure (90-120 psi).

13. Check the argon gas cylinder outlet pressure at the tank and the argon gas operating gauge pressure (8-10 psi).

14. Make sure the Vacuum light on the Quattro Micro is green.

15. Prime (clean) the autosampler syringe to get rid of large bubbles

a. Press F2 (Clean Syringe) on the autosampler controller.

b. Wash station is set to Wash 1. Press center button of the dial to accept or turn the dial to select Wash 2 and press center of the dial to accept.

c. Select 5 wash cycles by turning dial to 5 and pressing center button. Autosampler syringe will wash 5 times.

16. Sonicate the sample cone. See Appendix A for a complete procedure distributed by PerkinElmer.

a. If instrument not already in standby mode, put it in in **Standby** mode by clicking **Press for Standby** on Tune page and turning off the API gas. <u>Collision gas remains on at all time.</u>

b. Remove the cover, unfastened the latches to open the ionization chamber doors.

c. Close off the vacuum by moving the vacuum isolation valve lever to the right.

d. Remove Teflon tubing from gas cone nozzle (for cone gas/curtain gas, nitrogen). Remove the reflector plate and place it on a clean surface.

e. Unscrew the 2 metal hex nuts and place it near the reflector plate.

f. Remove cone cover plate and place it near the reflector plate.

g. Carefully slide out the cone assembly to avoid dropping or tumbling out of sample cone (inner cone).

h. Separate cone gas nozzle (outer cone) from sample cone and remove O-ring from sample cone with provided cushioned tweezers. The tip of the sample cone can be damage easily, thus, take care not touch the tip. Place the O-ring in the same clean area. Do not sonicate the O-ring.

i. Allow 5-10 minutes for the cones to cool.

j. Place sample cone and outer cone in two separate 50 or 100 mL beakers filled with enough methanol and DI water (50:50) to cover the top of the cones, approximately 40ml for the 50mL beakers.

NOTE: Use 50% methanol (HPLC grade) and 50% DI water (HPLC grade) for the Sonication.

k. Sonicate the beakers containing the cones for 25-30 minutes.

l. Take both cones out of the beakers. Rinse them with methanol inside and out. Hold the sample cone by the base using provided cushioned tweezers to avoid damaging sample cone's tip.

m. Allow the excess methanol to evaporate. Do not wipe or rub the sample cone. Wipe inside of the chamber with Kimwipe wet with Methanol. Avoid touching the capillary.

n. Carefully, place the cone assembly back in the instrument. Seat the cones cover plate properly. Screw back the hex nuts. Put the reflector plate back in.

o. Reconnect the Teflon tubing and then turn the vacuum isolation valve lever to the left.

p. Make sure the ion chamber O-ring in the door is not loose or cracked.

q. Close the chamber door with the side latches.

r. Put the cover back on.

17. Prime the LC pump and purge the lines (while the cones are sonicating).
Do this only after completion of Steps 4 – 6.

a. Open the Inlet Method screen.

b. Select **File/Open** and click on the **Purge.w25 or Purge.PME file**.

c. Click on the **Load Method** icon from the Initial Conditions tab on the toolbar to start the pump.

Note: Make sure the in-line Filter is disconnected from the short yellow peek tubing.

d. Make sure the flow rate is set at 2 mL/min and the flow solvent line is free from air bubbles. Run the pump 1-3 minutes.

e. Click on the faucet icon to stop the pump.

f. Connect the **in-line Filter** assembly to the short yellow peek tubing and let the pump run for 1-3 minutes. Confirm that flow rate is set at 2 mL/min. Check for leaks.

g. Stop the pump by clicking on the faucet icon and connect the long yellow peek tubing from the capillary to the filter assembly.

h. Place couple of Kimwipes in the ionization chamber in order to avoid pooled or over flow of solvent during the purging.

i. Click on the faucet icon on the toolbar to start the pump. Let the pump run 1-2 minutes.

j. Check that the spray from the capillary is straight and steady at a flow rate of 2 mL/min.

NOTE: Do not adjust the capillary angle position. If there is any problem with the spray, please call Proxy for service.

k. Check and record the pump pressure on the maintenance log while the pump is priming. Pump pressure is found on the Status icon of the Inlet Method screen.

l. Stop the pump by clicking on the faucet icon on the toolbar after running for 1-2 minutes.

m. Wipe and clean the ionization chamber with methanol and a Kimwipe.

n. Tandem Mass is a very sensitive instrument. Dust particles can easily clog the syringe, injection port, and the ionization source. Thus, it is necessary to perform daily cleaning around the MSMS instrument prior to starting the analysis.

H. MS/MS Analysis

1. Fill out a checklist for each assay.

2. Click Open the following icons:

a) MS Tune → Open **API Gas** (Nitrogen Gas; Atmospheric Pressure Ionization). Collision Gas (Argon gas) must be on all the time.

b) MS Tune Method → Press **Operate** (look at the box to turn green). Monitor the operate light on the MS/MS to ensure it lights up green.

c) Inlet Method → Open the pump (NeoBase_AAAC_ND.W25 or NeoBase_AAAC_ND.PME) → Load Method → Check the flow rate, which should be 0.200-0.240 mL/min and the pressure is >24 psi.

3. Wait for at least 10-15 minutes in order for the MS/MS to reach equilibrium. For consistency, record the Vacuum Gas Pressure $(2.7 \pm 0.5)\ e^{-3}$ when well A03 is finished sampling.

NOTE: The time for the equilibrium stage varies from instrument to instrument.

4. Click on the **Open Sample List** Icon on the menu bar, and highlight the tray by clicking on the upper left corner of table that is to be run and click **Open**. This will display the sample list for the selected tray on the screen.

5. Start the tray by clicking on the **Run/Play** button on the toolbar. The Start Sample List Run box will appear. Make sure that the first two boxes, **Acquired Sample Data** and **Auto Process Samples** are checked. Under the **Run** heading, make sure that the correct number of samples (e.g. 1-45; 1-96) are listed and click **OK** to start the run.

6. Click **Chromatogram** to open the chromatogram window. Click on the **Clock Icon** in the top menu bar of the chromatogram window to show the current sample being acquired. Make sure that the **TIC** shape and intensity (**>1.0e6**) of the **IS** wells are acceptable.

7. In order to view the results for a specific tray, open the **NeoLynx Browser** window. Open the file for the current tray being sampled. In the menu view, select **autorefresh**.

8. To add more trays to the run, repeat steps 4 and 5 and put them in **Queue**.

9. Click **Queue Icon** on the toolbar to verify that all the trays to be run are present. Each sample takes close to 2 minutes. An entire 96 well tray takes about 3 hours to complete.

10. At the end of the run, minimize the **MassLynx** screen and click **MS/MS Data Transfer** to transfer the results to Specimen Gate for review. Click→ **Yes** when the message appears asking if you would like to transfer the completed tray. Confirm tray ID before transfer.

NOTE: Do not transfer data while the MS/MS run is in progress. It will transfer the current run which will have incomplete data.

If a lab has two MS/MS systems, each system must be used at least 3 days a week.

I. Daily Shutdown

1. Manual Shutdown

a. Press **Standby** on the Tune Page after the last tray has been run**.**

b. Desolvation gas temperature will drop down to 25 – 30 ºC from 350 ºC.

c. Turn the faucet off in the inlet method.

d. Turn off API (Nitrogen) gas. The Collision (Argon) gas remains on at all times.

2. Automated Shutdown Editor

a. The **Shutdown Editor** is used to shutdown the system (LC pump and mass spectrometer) automatically after the last tray has been run.

b. The **Shutdown Editor** can be selected in MassLynx under Instrument. Select **Edit Shutdown** or **Setup**.

c. Select **Enable Shutdown after Batch**. Click on **Browse** to choose the file: Shutdown_daily.acl.

d. Under the **Auto Control Tasks** tab, check that these tasks are listed for this file: LC pump off (post delay 30 sec), Standby, and Source Gas Off (ESP).

e. In the Shutdown tab, under the Shutdown Time heading make sure that the Shutdown time after batch or error is 10 minutes.

f. Close the window.

J. Repeat Testing of Newborn Specimens

1. Rules for Repeat Testing:

a. If a run or tray is prevented from release, repeat the testing for that run or tray.

b. Reasons for repeat of a tray

1) When one or more controls are outside the ±3SD limits and the 80% QC rule failed. In order to pass the 80% QC rule, twenty one of the thirty nine analytes must pass (80% of the total analytes).

2) When a pair of controls is outside the ±2SD limits and the 80% QC rule failed.

3) When the TIC intensities of multiple analytes (>10) of patient wells are below the acceptable limits.

4) Tray demonstrates significant low or high QC bias.

5) When positions of patient samples are switched as indicated on the plate map.

6) When there is a known laboratory error.

7) When ≥ 15 patient wells are blue (presumptive positive).

c. Reasons for repeat of a single specimen may include

1) Clog detected in the sample line.

2) Missing blood spot in the well (no data).

3) Autosampler failed to sample a specimen (no data).

4) The intensities of more than 5 internal standards are below the acceptable limits.

5) The intensity of the MRM TIC spectra is below the acceptable limits.

6) The shape of the MRM TIC spectra is unacceptable.

7) The concentrations of more than four analytes in the sample well are above the established cutoff limits.

8) Four or more analytes for a well are scored blue and indicative of four or more disorders.

9) Analyte concentration is greater than the linearity range is "Panic Values". If the tray passed QC and is released, you must call the appropriate ASC to report that the newborn's result is greater than the linearity range for the analyte, and the sample is being repeated. The repeat result will be called again as soon as it is available. The timely in reporting these extremely high results may mean differences between life and death for some newborns.

10) Analyte concentration is less than the low detection limit.

11) If C3 ≥ 10.0 and C3/C2 ratio is within normal limits, prevent the sample and repeat on a different MS/MS, if possible, to confirm.

12) If a result for a single specimen is prevented from a released tray, run that specimen as a repeat on the next run or the next day's run (whichever comes first) along with new samples.

13) A known laboratory error.

d. Print the Repeats List generated by the NAPS laboratory immediately after the supervisor's review and release. All newborn screening results which were prevented from release are listed and must be tested no later than the next day. Refer to the MSMS Newborn screening Supervisor's Daily Review and Release Protocol for instructions on how to print the repeat list.

e. Print the Repeats Requested by GDL each morning. The list is downloaded to the screening laboratory after data review at GDL. Those listed must be included in that day's testing.

NOTE: If repeat is not needed and the result is released by the supervisor, the specimen will drop off the list. If prevented or not tested, the specimen will stay on the list.

K. Reporting Results

1. Identify the ASC to call and report the following identified by Specimen Gate as below. Add comment that results was called.

a. Overall Positive either for (AA or AC) or both

b. AA or AC analyte is marked as Urgent

c. Analytes is marked as Positive

d. Panic Values are values of Amino Acids and/or Acylcarnitines that are greater than linearity range.

2. Do not report "Review" and "Out of Range".

3. Report results in µmol/ L for all analytes.

4. Follow the Newborn Screening Accession and Reporting at the NAPS Lab protocol for reporting presumptive positives.

a. Review and release MS/MS trays at the Supervisor's PC.

b. Right Click on the positive well.

c. Click on **Presumptive Positive Report** tab.

d. Click on the Printer Icon for printing the result.

e. Call the area coordinator to report positive along with the disorder(s) and concentration of the analyte(s).

f. Complete the Confirmation of Contact (C of C) in SIS.

g. Enter the reporting information on the MS/MS Presumptive Positive log.

h. FAX the positive specimen print out (containing the disorder and analyte concentrations) along with at a copy of TRF to the coordinator.

NOTE: If a sample is positive for only one group (AA or AC) of analytes and not for the other, report out disease related to that group of analyte(s) only.

L. Setting up the run when there are Repeats

1. Repeat Tray(s)

A prevented tray is repeated by the next day and tested as the first tray of that day's run.

2. Repeat Individual Specimen(s)

a. A prevented sample is repeated on the next run or first run of the next day and tested as the first sample at the beginning of the tray following CT sample, before new samples.

b. Repeat individual specimen that has analyte concentration higher than (>) the high value of its linearity range:

1) Do not dilute.

2) Repeat the specimen on the run or by the next day.

3) Release the result like other repeated specimen.

c. Repeat individual specimen that has analyte concentration less than (<) the low value of its linearity range.

Note: If repeated results still higher or lower than the linearity range values, currently, the system will report the obtained values.

M. Inventory

Refer to AutoDELFIA for the Determination of TSH and 17OHP Protocol.

N. Preventive Maintenance

The analyst records the preventive maintenance performed in the appropriate log (see attached). The supervisor or designee reviews and signs each preventive maintenance log periodically.

1. **Puncher**

a. Weekly

1) Remove the punch head.

2) Remove the piston from the punch head and clean with methanol.

3) Clean the punch head cylinder with methanol.

4) Wipe the inlet shaft with methanol using a cotton swab.

b. Monthly

1) Remove and clean the punch head. Put it in storage.

2) Install the 2nd punch head.

3) Wipe the puncher unit with a clean, Methanol moistened wipes.

2. Incubator/Shaker

a. Weekly

Wipe the tray holders and outside of the incubator/shaker with methanol moistened wipes.

3. Sample cone and cone gas nozzle cleaning

a) Place the sample cone and cone gas nozzle (not the O-ring) in two separate beakers with HPLC grade methanol: water (1:1).

1) Use a clean, designated beaker for each cone; do not clean beakers with surfactants or other materials known to cause contamination to the mass spectrometer.

2) Use the cushioned tweezers provided by PerkinElmer for handling the cones for better grip.

b) Sonicate the beakers in an ultrasonic bath for 25-30 minutes.

c) Remove the sample cone and cone gas nozzle from the methanol/water mixture and place them into another beakers containing 100% methanol.

d) Place the beakers in an ultrasonic bath for 10 minutes.

e) If either part of the cone gas assembly is stained with dark stains, do the following for cleaning.

1) Rub the outer cone gas nozzle with moistened Methanol and DI water on a soft, lint-free wipe (such as a Kim-wipe), but NOT the inner sample cone.

2) Soak/sonicate in a 45% methanol (HPLC grade), 45% DI water (HPLC grade), and 10% formic acid solution for 30 minutes (Warning: Use with care with formic acid; adding under fume hood and use personal protective equipment.)

3) Rinse by sonication in HPLC grade DI water for 20 minutes.

4) Sonicating in 100% HPLC grade methanol for 10 minutes.

f) Carefully remove the parts from the beaker, blow-dry them using inert oil-free gas, or, alternatively, the parts may be placed on lint-free towels and allowed to air dry.

Reassemble the cone gas nozzle, O-ring, and sample cone and then place them back in the ionized chamber, secure them with the retainer plate and the two screws.

4. Sonicator

a. Monthly

1) Empty water from the Sonicator.

2) Wipe inside of sonicator with a Kimwipe moistened with distilled water.

3) Refill the Sonicator with distilled water to the Water Level line.

4) Run the Sonicator for 15 minutes without the sample cone beakers.

5. Apricot Pipettor

a. Daily

1) Change wash water.

2) Oil Apricot tip head in lubrication box.

3) Change Apricot tips.

4) Remove the metal Apricot pipette tip head after its use for the day.

b. Monthly

1) Add mineral oil to lubrication box.

2) Perform well-to-well precision.

a) Take out the following supplies:

- hCG Tracer (from used DELFIA kit) and Enhancement Solution
- 2 Apricot Reservoirs (supplied by PerkinElmer; dedicated for A&P only, do not use for clinical testing)
- Plates cover From the PE Tomtec A&P Kit,
- Microtitration Plate with “Plate 27µL and 200 µL” barcode

- Reagent Cassette label with "200 µL Precision" barcode
- Clean glass beaker or flask.

b) Add 100 µL of hCG tracer to 150 mL of Enhancement Solution in a clean beaker or flask. Cover with parafilm. Swirl gently to mix for a homogeneous solution. After mixing, wait (15-20 minutes) until solution is free of air bubbles or foam. For 75 µL, the target counts should be around 120,000 - 200,000 per well.

c) Add diluted tracer to one of the Apricot A&P reservoirs. Take care to minimize as much foam or bubbles as possible. Place this reservoir on station 4 of the Apricot (Apricot tips should be loaded).

d) Fill the second Apricot A&P reservoir up to about ¼ to ½ with undiluted Enhancement Solution and place it on station 3 of the Apricot.

e) On the Apricot PC, click **File → Open**. Select "Well Precision 75µL-eu & 125 µL enh" (eu = tracer + Enhancement solution and enh = only Enhancement solution).

f) Place a clean microtitration plate on station 1. Click **Run** after done with the three checks.

g) Cover the tray immediately after solutions have been dispensed. The tray should be counted within a few minutes of pipetting. Tab gently to break the air bubbles in all the wells before load the plate on AutoDELFIA.

h) Measure precision using AutoDELFIA.

1. Place the reagent cassette with the *200µl Precision* bar code in position 1 of AutoDELFIA reagent rack area.

2. From **GDLB-PNS & NBS Program Menu**, click on **Instrument Programs**, then select **AutoDELFIA Workstation**. Click **OK**, then **Yes**. This initialization process takes about 15-20 minutes.

3. Skip step 2 above and go to step 4 below if the smiley face/Start arrow icon is already active on the screen.

4. Click on the lighted smiley face/Start arrow icon under **My task**. If it is not active, click **Loading** and select one of the unload options. Remove the used plate on the loader if one is present. Click **Cancel,** then **OK, OK.** The smiley face should now be active, click on it.

5. Type in your user name in the log on box, click **OK** or press Enter key.

6. Loading Wizard appears. Click **Next**, then **Next**. If no response on the second **Next**, click on **Unload old samples**. Remove sample racks as needed, and then click **Next** again.

7. Click on **Create New** tab**.** Click on the **+** sign next to **Service** option. Double click on **910 Tomtec Prec 200**, then **Next.**

8. Under **tube types**, click on the drop down arrow and select **Man. Pipetted** option.

9. Under **code**, type: **1**, and under **number of samples to add**, type: **96**. Then click on **Add Samples** tab.

10. Click **Next** (seven times) until **Reagent consumption** screen appears. Click **OK**, see **Liquid consumption** box. Check to make sure liquid reagent levels in the diagram are okay. Replace, replenish if needed. Click **OK.** Now the instrument scans the reagent bar code and the loader comes out.

11. Load the prepared tray on the tray loader with the bar code facing the mirror. Press the lighted **IN/OUT** button.

12. Click **Next** when prompted, see **Wizard completed** message. Click **Start** and see **Process initialization**. Stand by the instrument as the message indicated. Okay to walk away from the instrument when **Schedule** box appears. The run will take about 10-12 minutes and the instrument will take additional 10-15 minutes to wash/rinse the tray. One can print the results of the run as soon as the run is finished and while the instrument is doing the wash/rinse process.

13. To print the result, click on **Print A&P Run**, then click on **Print last 200µl run**.

14. Click on **Close** when it lets you.

15. Click on **AutoDELFIA Workstation** box to activate it. **C**lick on **Loading** to unload your plate and remove it when the loader comes out.

16. Click **Cancel**, **OK**, then **OK** to abort previous run.

17. Rinse the finished plate thoroughly with DI water, even though, it was rinsed by AutoDELFIA. Make sure the plate is completely dry before put it away for next uses.

18. For well-to-well precision at 75 µL, acceptable CV is ≤5%, and all wells should be within 15% of the mean count. The MultiCalc printout will give the calculated mean and %CV. It will flag any wells outside the limits. If results are outside of these limits, repeat, troubleshoots. Call Proxy for assistant if needed.

3) Perform accuracy

a) Take out a clean clear Plate. Weight the empty clean plate.

b) Make sure there is sufficient distilled water in the Apricot wash reservoir. Measure water temperature at room temperature.

c) Place the weighted clear plate at the appropriate Apricot station as indicated by the program (the wash reservoir should be at station 4 and the Apricot tips should be loaded).

d) Click File/Open. Select and open the **Service Accuracy Test for** 75 **µL** program. Click **Run**. 75 µL of distilled water will be dispensed into the tray at station 1.

e) Weight the filled plate and calculate the average volume per well with temperature correction. Acceptable range for these volumes is 72.75 – 77.25 µL.

f) See Appendix B for Water Density conversion chart based on selected temperatures. Temperature correction calculation:

(Water weight in g) / 96 wells / (density in g/mL from temperature table) x 1000µL / 1mL = average volume / well in µL.

Example: Temp. = 23.0°; density of water at 23.0° = 0.9975694g/mL

(37.59g-30.57g)/96/0.9975694g/mLx1000µL/1mL = 73.30µL (corrected)

NOTE: If the well to well precision or accuracy values are not within acceptable ranges, repeat, if still outside limits, call Proxy for service.

6. Quattro micro

a. Daily (Refer to VII.G.8-17.)

b. Weekly

1. Sonicate sample cones with a 10% Formic Acid/Methanol solution, i.e., add 4 mL of Formic Acid to ~36 mL of Methanol for 25-30 minutes.
2. Rinse well in distilled water; sonicate the cones for 15 minutes in 100% methanol to remove all residual traces of formic acid.
3. Ballast vacuum pump

a) Quattro micro can be either in Standby or Operate mode and gases can be on or off.

b) Turn the gray pump ballast knob, located behind the vacuum pump demister, counterclockwise to open.

c) Keep it open for 15 minutes to allow the oil collected in the demister to return to the pump.

d) Turn the knob clockwise until closed.

c. Monthly

1. Change the yellow and red PEEK tubings. The tubing lengths should be 5 feet for both. After the ends of the tubings have been trimmed with the PEEK tubing cutter, turn on the pump flow to check the flow stream. The flow should be even and straight for both ends.
2. Check oil level and color in vacuum pump. If oil level is less than half full or oil color is dark, contact Proxy for Technical Support and service.

d. Quarterly and Bi-annual

Quarterly and Bi-annual maintenances are performed by PerkinElmer Technical Service Engineers.

7. Pipettes

Perform accuracy and precision quarterly.

a) 100 - 1000 µL single-channel pipette

1) Weigh five empty weighing cups.

2) Set the pipette to 100 µL and dispense 100 µL DI water into each of the five cups.

Note: Use a thermometer to measure the water temperature at room temperature.

3) Re-weigh the five cups containing 100µL DI water.

4) Calculate the volume dispensed using the formula:

(Final weight – tare weight) x 1000µL/1mL / density of water in g/mL (Appendix B)

5) Determine the average volume and %CV.

6) Repeat steps 1-5 with pipet setting at 1000µL.

7) The acceptable results must meet the following criteria.

Volume	Average (±2%)	CV= (SD/AV)x100%
100 µL	98 - 102	1.5%
1000 µL	980 - 1020	1.0%

b) 20 -200 µL single-channel pipette

1) Weigh five empty weighing cups.

2) Set the adjustable single-channel pipette to 100µL and dispense 100µL distilled water into each of the five cups.

Note: Use a thermometer to measure the water temperature at room temperature.

3) Re-weigh the five cups containing 100µL of DI water.

4) Calculate the volume dispensed with the formula:

(Final weight – tare weight) x 1000µL/1mL / density of water in g/mL

5) Determine the average volume and %CV.

6) The acceptable results must meet the following criteria.

Volume	Average (±2%)	CV = (SD/AV)x100%
100 µL	98 - 102	1.5%

c) 50 - 300µL multi-channel pipettes

Perform well-to-well precision and accuracy for 100µL (and 75µL if the pipettes are also use for this volume)

1) Perform well to well precision

a) Take out the following supplies:

- hCG Tracer (from used DELFIA kit)
- Enhancement Solution Apricot Reservoir, supplied by PerkinElmer, dedicated for A&F only, do not use for clinical testing
- Plate cover From the PE Tomtec A&P Kit
- A clean Microtitration plate with special barcodes (can be the same plate which is used for Apricot precision)
- A special reagent cassette label with 200 µL Precision
- A clean beaker or Erlenmeyer flask big enough to contain the solution.

b) Add 250 µL of hCG tracer to 100 mL of Enhancement Solution into the clean beaker or flask. Wrap with parafilm and swirl gently to mix well for a homogeneous solution. After mixing, wait until the solution is free of air bubbles and foam, about 15- 30 minutes. If the solution still has bubbles or foam, use the solution in such a way that the bubbles or foam do not get transferred into the wells while pipetting.

c) Dispense 100µL (and or 75µL) of the homogenous solution into the clean 96 well microtitration plate labeled as "Plate 27µL and 200µL". Using the same multichannel pipette that was used to dispense the prepared solution but with clean tips, dispense 100µL (or 125µL) of undiluted Enhancement Solution to bring each well volume to 200µL total.

- Apricot Pipettor has programs to dispense the undiluted Enhancement solution to the plate. For doing pipette precision at 100µL, select "Well Precision Dispense 100µL enh" program. For doing pipette precision at 75µL, select "Well Precision Dispense 125µL enh" program. Position the plate and the reservoir accordingly.
- Check the microtitration plate to be sure there are no air bubbles in each wells after adding tracer-Enhancement solution and undiluted Enhancement solution. Presence of air bubbles can cause falsely high %CV. I f air bubbles are present, gently tab the plate to get rid of them before load the plate on AutoDELFIA.

Note: Each laboratory is responsible for doing accuracy and precision on all the pipettes in use and must include the volume that the pipettes are used for.

d) Measure using-AutoDELFIA.

1. Follow instruction from Section VII.N.5.h.

2. The acceptable well-to-well %CV specified by GDL is ≤ 5%. If results are outside these limits, repeat and if still outside the limits, call Proxy.

e) Wash the Apricot A&P reservoir thoroughly under running water or DI water and then soak in DI water for 5-10 minutes and thoroughly rinse again with DI water before air dry it.

f) Acceptable % CV for 100µL well-to- well precision is ≤5% and all wells should be within 15 % of the mean count.

2) Perform accuracy for 100µL (and 75µL as needed)

a) Weigh five clean 96-well trays provided by Perkin Elmer for A&P procedures only. Or use one clean tray and tap to dry on paper towels between weighings.

b) Set the 50-300 µL adjustable multi-channel pipette to 100 µL (or 75µL).

c) Dispense 100 µL (or 75µL) of distilled water into each well of a tray.

Note: Use a thermometer to measure the water temperature at room temperature.

d) Re-weigh the trays containing DI water and use the formula below to determine the average volume per well.

(Final weight -tare weight)/96 x 1000µL/1mL / density of water in g/mL

e) Repeat five times if only use one tray.

f) Determine the average volume per well and %CV.

g) Results are acceptable if data meet the following criteria:

Volume	Average (±2%)	CV = (SD/Avg)x100
100µL	98-102	1.5%
75µL	73.5-76.5	1.5%

O. Waste Handling

1. Discard the biohazard waste (specimens, trays, transfer pipets, pipet tips, adhesive plastic covers, heat sealing film, aluminum foil, and solution basins) into the biohazard bags.

2. Collect methanol liquid waste in plastic or glass waste containers with a label on each container. Each label should list the type of waste, collection start date, the date emptied, and the initials of the person who empties the container.

3. Be sure that all waste containers are on a secondary tray in case there is a spill or leak.

4. Check the waste collection containers daily. Empty the containers once they become ¾ full.

5. Do not discard any chemicals down the drain.

6. To dispose of the liquid hazardous waste

a. Ensure the liquid is in a proper disposable container, 1 gallon plastic jug with screw top lid.

b. Fill out and attach a Hazardous Waste label on the outside of the container. The label should list: Contents, Generator (CA DHS, GDL and address), Hazard Category (Flammable, Corrosive, Toxic, Reactivity, Other), Contact Number (person disposing waste and telephone number), and Start Date (start date of collection).

c. Fill out a Hazardous Waste and Material Disposal Form (on file in lab or contact an Industrial Hygienist). This form will be given to the Industrial Hygienist when the waste is disposed.

NOTE: Handle the waste per your local, county and state regulations.

VIII. Calculations

Software automatically calculates concentration of analyte based on signal of the analyte relative to the signal and known concentration of the IS for the analyte.

IX. Reporting Results

Prior to sending results to GDL for release, the results are reviewed for validity. The validity of results is judged by running a quality control program that monitors the performance of the MS/MS system and day-to-day performance of the method.

A. Monitor the Performance of the MS/MS System

Two system controls, low (CL) and high (CH) formulated with amino acids and acylcarnitines, provided with the reagent kit, are analyzed with each run. The controls are

placed in the beginning of the tray in positions 4 and 5, and at the end tray in positions 95 and 96. The system controls are flagged automatically by the software if outside ±2SD limits and if outside ±3SD limits. The performance of the MS/MS system is judged acceptable if the results meet the limits set by GDL. Specifically, the results of system controls together with tray controls assist in determining the source of error, such as degradation of internal standards and reagents, instrument malfunction, operator error, incorrect reconstitution, and sampling error.

The supervisor may consult with GDLB to determine the source of error, interpret its meaning, and take corrective action.

B. Monitor Day-to-Day Performance of the Method

A blood spot tray control (TQC) formulated with amino acids and acylcarnitines is provided by GDLB with quality control action limits. Two TQC's are analyzed on each tray of newborn specimens. The TQC's are placed in positions 6 and 94 on the tray. The results of the TQC's are judged based on the action limits set by GDL. The tray controls are flagged automatically by the software if outside ±2SD limits and if outside ±3SD limits.

If the tray control falls outside the acceptable limits, the supervisor in consultation with GDLB will determine the source of error, interpret its meaning, and take corrective action.

NOTE: Position E1 for each tray is designated for C (control) P (proficiency). This CP is not scored by the software. The control is used for GDLB purpose only.

C. Sending Results to GDL

The Supervisor will review and score each tray as green (release), yellow (warning) or red (prevent) based on quality control rules. The analyst and the supervisor are required to enter relevant comments regarding the instruments and the assay on the checklist and in the supervisor's PC, which will assist GDL to further evaluate the tray data prior to releasing. After supervisor's review, the data is transmitted to GDL using **Transfer Now** or overnight. The completed checklist is faxed to GDL by 10:00 am the next morning.

NOTE: During reviewing, if consecutive blue colored patient wells have same analytes and/or disorder(s) flagged, enter comment at these wells level whether or not these wells are related, e.g., siblings, twin A, B, or C, etc. This will assure reviewer/releaser at GDL that these wells were not accidentally punched repeatedly from the same patient card.

X. Procedure Notes

A. Analysis of an Interrupted Tray

1. If for some reason, the autosampler stops in the middle of sampling a tray, the analyst must analyze the rest of the samples within 8 hours after reconstitution.

2. When an error has occurred, the sample list is Paused (the Pause button on the toolbar is depressed). Press the Pause button to un-pause the sample list and resume the run.

3. If the sample list was stopped (the Stop button was pressed), the sample list is automatically removed from the queue. Highlight the samples in the sample list that need to

be sampled and press **Run/Play** icon to restart the run (the sample list will appear in the queue).

B. Manual Preparation of the Worklist

1. Open MassLynx. A new sample list screen will appear (only one sample line will appear and each field will be empty).

2. For the first sample, fill in the file name: Tray barcode#_lab_accession date_01 (01 indicates the well location for the first sample).

3. Double click in the MS Tune File column. Select **NeoBase_AAAC_ND.ipr**.

4. Double click in the MS Method column. Select **NeoBase_AAAC_ND**.exp.

5. Double click in the Inlet file column. Select **NeoBase_AAAC_ND**.

6. In the Well column for the first sample, indicate the tray # and well # (i.e. 1:1 indicates tray 1, well 1).

7. For all samples, enter 30 (µL) in the Inject Volume column.

8. Double click in the Process column. For all samples, select NeoLynx.

9. Double click in the Parameter file column. Select **NeoBase_AAAC_ND.ntf**.

Note: The Parameter is the current ***kit*** lot number that is being used.

10. Above the sample list, under Samples select Add.

11. Enter in the number of samples to add after the first sample that was entered. Click OK.

12. Most fields for each sample will be filled in.

13. Fill in the Sample ID column for each type of sample: IS (internal standard only wells), CL/CH/CT (controls), and PT (patient sample).

14. Double click in the Sample Type column to select for each sample: Blank (for IS), QC (for controls), and Analyte (for patient samples).

15. Check to ensure that these fields are filled in appropriately for each sample: File Name, Sample ID, MS Tune File, MS Method, Inlet File, Well, Inject Volume, Sample Type, Process and Parameter File.

16. To fill a column down with either the same value or sequential numbers, select the first cell then drag down to the last cell (those cells will be highlighted). Right click and select either Fill Down (to fill the same value into each cell; i.e. Inject Volume will be 30 µL for every sample) or Fill Series (to fill in sequential values; i.e. Well # will increase for each sample). Remember to indicate the tray # before the well # in the Well column (separated by a colon; i.e. 1:1, 1:2,…)

XI. Biochemical Tables

Table 1

A. Masses of Biochemical Markers and Their Internal Standards

Function	Primary Markers in Blood		Internal Standards	
	Analyte Name	**Analyte Mass (AMU)**	**Internal Standard Name**	**IS Mass (AMU)**
MRM -1				
	Alanine	90.0	D_4-Alanine	94
	Arginine	175.1	D_4, ^{13}C-Arginine	180.1
	Citrulline	176.1	D_2 -Citrulline	178.1
	Glycine	76.0	^{15}N, 2-^{13}C-Glycine	78.0
	Isoleucine	132.1	D_3-Leucine	135.1
	Leucine	132.1	D_3-Leucine	135.1
	Methionine	150.1	D_3-Methionine	153.1
	Ornithine	133.1	D_6-Ornithine	139.1
	Phenylalanine	166.1	$^{13}C_6$-Phenylalanine	172.1
	Proline	116.1	$^{13}C_5$-Proline	121.1
	Tyrosine	182.1	$^{13}C_6$-Tyrosine	188.1
	Valine	118.1	D_8-Valine	126.1
	Succinylacetone (SA)	155.1	$^{13}C_5$-Methylpyrazolyl-propanoic acid (MMP)	160.1
MRM -2				
	C0	162.1	D_9-C0	171.1
	C2	204.1	D_3-C2	207.1
	C3	218.1	D_3-C3	221.1
	C3DC	248.1	D_3-C4	235.2
	C4	232.2	D_3-C4	235.2
	C4OH	248.1	D_3-C4	235.2
	C4DC	262.2	D_9-C5	255.2
	C5	246.2	D_9-C5	255.2
	C5:1	244.2	D_9-C5	255.2

	C5OH	262.2	D_9-C5	255.2
	C5DC	276.1	D_6-C5DC	282.1
	C6	260.2	D_3-C6	263.2
	C6DC	290.2	D_6-C5DC	282.1
	C8	288.2	D_3-C8	291.2
	C8:1	286.2	D_3-C8	291.2
MRM -3				
	C10	316.2	D_3-C10	319.2
	C10:1	314.2	D_3-C10	319.2
	C10:2	312.2	D_3-C10	319.2
	C12	344.3	D_3-C12	347.3
	C12:1	342.3	D_3-C12	347.3
	C14	372.3	D_3-C14	375.3
	C14:1	370.3	D_3-C14	375.3
	C14:2	368.3	D_3-C14	375.3
	C14OH	388.3	D_3-C14	375.3
	C16	400.3	D_3-C16	403.3
	C16:1	398.3	D_3-C16	403.3
	C16OH	416.3	D_3-C16	403.3
	C16:1OH	414.3	D_3-C16	403.3
	C18	428.4	D_3-C18	431.4
	C18:1	426.4	D_3-C18	431.4
	C18:2	424.3	D_3-C18	431.4
	C18OH	444.4	D_3-C18	431.4
	C18:1OH	442.4	D_3-C18	431.4

Table 2

B. MS/MS Cutoff for Amino acids and Succinylacetone

Analyte	Type of Analyte	Cutoff in (µMole)	Cutoff Type
Alanine	AA	1000	High
Arginine	AA	50	High
Citrulline	AA	60	High
Citrulline	**AA**	**5**	**Low**
Glycine	AA	…	…
Leucine/Isoleucine	AA	250	High
Methionine	AA	100	High
Methionine	**AA**	**8**	**Low**
Ornithine	AA	800	High
Oxoproline	AA	…	
Phenylalanine	AA	155	High
Proline	AA	1500	High
Tyrosine	AA	850	High
Valine	AA	…	…
Arg/Orn	AA Ratio	1.4	High
Cit/Arg	AA Ratio	6.0	High
Phe/Tyr	AA Ratio	1.5	High
Leu/Ala	AA Ratio	1.3	High
Val/Phe	AA Ratio	3.5	High
Succinlyacetone	Ketone	4.5	High

MS/MS Cutoff for Free and Acylcarnitines

Analyte	Type of Analyte	Cutoff in (µMole)	Cutoff Type
C0	**AC**	**7.0**	**Low**
C0	AC	125	High
C2	**AC**	**11**	**Low**
C2	AC	80	High
C3	AC	6.3	High
C3DC	AC	0.38	High
C4	AC	1.7	High
C5	AC	1.0	High
C5:1	AC	0.6	High
C5OH	AC	0.9	High
C5DC	AC	0.6	High
C6	AC	0.95	High
C8	AC	0.6	High
C8:1	AC	0.7	High
C10	AC	0.65	High
C10:1	AC	0.45	High
C12	AC	2.0	High
C14	AC	1.2	High
C14:1	AC	0.8	High
C14OH	AC	0.2	High
C16	AC	10	High
C16:1	AC	1.4	High
C16OH	AC	0.10	High
C18	AC	4	High
C18:1	AC	7	High
C18OH	AC	0.10	High
C18:1OH	AC	0.10	High
C3/C2	AC Ratio	0.3	High
C0/(C16+C18:1)	AC Ratio	75	High
C16OH/C16	AC Ratio	0.07	High
C3DC/C10	AC Ratio	4.0	High
C5/C3	AC Ratio	0.45	High
C5DC/C3DC	**AC Ratio**	**0.70**	**Low**

Table 3

C. Analytical Linearity Range of Amino Acids, Succinylacetone, and Acylcarnitines in Dried Blood Spots

Analyte	Linearity Range (μM)
Alanine	4.5- 4090
Arginine	0.6 - 3721
Citruline	4.8 - 1683
Glycine	50.4 - 4487
Leucine	1.3 - 2545
Methionine	2.5 - 1185
Ornithine	0.6 - 3771
Phenylalanine	0.3 - 2341
Proline	4.7 - 3659
Succinylacetone	0.24 - 158.1
Tyrosine	1.2 - 2816
Valine	0.6 - 2358
C0	0.2 - 2274
C2	0.2 - 735
C3	0.03 - 88
C4	0.07 - 59.81
C5	0.04 - 59.1
C5DC	0.08 - 28.85
C6	0.08 - 61.54
C8	0.02 - 35.2
C10	0.04 - 28.86
C12	0.04 - 42.74
C14	0.02 - 41.81
C16	0.1 - 107.3
C18	0.04 - 32

Table 4

D. Disorders Detected by MS/MS and their Biochemical Markers

Disorder	Abbreviation	Primary Analyte Elevation	Secondary Analyte Elevation	Urgent Disorder
CPT-II, CAT Deficiency	CPT-II	C16	C18, C18:1	
LCHADD, TFP Deficiency	LCHADD	C16OH, C16OH/C16, C18OH and C18:1OH	C14OH, C16	YES
VLCADD	VLCADD	C14:1	C14	YES
MCADD	MCADD	C8	C6,C10,C10:1	YES
IVA, 2MBCDD	IVA	C5[not C4] and C5/C3		YES
3MCC, MGA, HMG	3MCC	C5OH (not C5:1)		
BKT, 2MO3OHBCDD	BKT	C5OH	C5:1	
GA-I	GA-I	C5DC		YES
GA-II, EE	GA-II	C4, C5	C6, C8, C8:1, C10, C10:1, C12, C12:1, C14, C16, C16:1, C18, C18:1, C18:2	YES
SCADD, IBCDD	SCADD	C4[not C5]		
MCD	MCD	C3 and C3/C2 or C5OH		YES
PA/MMA	PA/MMA	C3 and C3/C2		YES
Malonic Aciduria	MAL	C3DC, C3DC/C10 and C5DC/C3DC (Low cutoff)		YES
Carnitine Transporter Deficiency	CTD	C0 ≤ Low cut off C2 ≤ Low cut off		
CPT1	CPT1	C0/(C16 + C18:1)		

Table 4 (continued)

Disorder	Abbreviation	Primary Analyte Elevation	Secondary Analyte Elevation	Urgent Disorder
Maple Syrup Urine Disease	MSUD	Leu , Leu/Ala and Val/Phe		YES
Citrullinemia (I/II) ASL Deficiency	CIT	Cit and Cit/Arg		YES
Argininemia	ARG	Arg and Arg/Orn		
HHH Syndrome Gyrtae atrophy, Hyperornithemia w/ Gyrate atrophy	HHH	Orn	Orn/Cit	
5- Oxoprolinemia	Oxopro	5- Oxopro		
Hyperprolinemia	Hypro	Pro		
Homocystinuria	HCY	Met		
Non–Ketotic Hyperglycinemia	NKH	Gly		
Phenylketonuria	PKU	Phe, Phe/Tyr		YES
Tyrosinemia	TYR	Tyrosine		
Tyrosinemia type I	TYRO I	Succinylacetone (SA)		YES

XII. Troubleshooting Guidelines

A. General Troubleshooting Guidelines

1. Do not overlook the obvious. Look for the simplest and most apparent cause and/or solution for a particular problem.

2. Rule out analyst induced errors. Consult protocols to review proper operation procedures and test methodologies.

3. Isolate and define the problem. The following categories of problems should be considered.

a. Regarding punching, was it a punching error by operator or jumping chads, sample(s) and/or tray should be re-punched.

b. Review internal standard reagent preparation, manual pipetting steps, temperature monitoring of heatblocks and incubator/shaker units, timing of each step, system set up steps were missed or performed incorrectly, etc.

c. Equipment performance, sample preparation equipment or MS/MS system, consult table listed below of common problems, symptoms and solutions for each piece of equipment.

d. Electronic problems, if PC components or cabling, call Proxy.

e. Software issues, if Specimen Gate or MassLynx software errors or worklist transfer errors (from puncher to MS/MS, importing into MassLynx, from MS/MS to supervisor's review and release PC), call Proxy.

4. Eliminate one variable at a time to clearly identify the problem.

5. Document the problem and solution. Maintain a Troubleshooting Log for analysts to describe the symptom(s), cause(s) and the solution(s) for future reference.

6. If analyst has performed all troubleshooting steps and problem continues to persist, call Proxy.

B. Troubleshooting Table

Equipment	Problem	Solutions
DBS puncher	No response.	Power is not turned on. Check electrical connections.
	PC not detecting puncher unit.	Puncher is not turned on. Puncher is not in Slave mode.
Apricot dilutor	No response.	Check that the power is turned on. Check electrical connections.
	Autosampler not aspirating and dispensing correctly or at all.	The Apricot tip head is not secured on Apricot tips. The Apricot tip head with the tips is not in the Apricot properly and is not tightened down properly. Check for cracked or bent tips.
Incubator/shaker unit	No response.	The power is not turned on. Check electrical connections.
	Tray holder not being detected.	The tray holder is not inserted properly. Clean metal contacts with a cotton swab moistened with methanol.
	Certain wells do not dry.	The dryer is not positioned towards the center of the tray.
Autosampler	No response.	The power is not turned on. Check electrical connections.
	PC loses connection with Autosampler.	Switch autosampler off then on.
	Liquid bubbling from the injection port.	Injector seals in injection port are clogged. Unclog/replace injector seals.
	Syringe leaking.	Syringe not tight in autosampler. Tighten syringe. Syringe is broken. Worn plunger. Replace syringe or plunger.
	Air bubbles in syringe.	Worn out plunger. Syringe is broken. Replace syringe or plunger.

Equipment	Problem	Solutions
	Autosampler not aspirating and dispensing properly or at all.	Fittings are not tight. Tighten/replace fittings. Autosampler tubing has leaks or clogs. Unclog/replace tubing. Make sure syringe is tight within the valve fitting. Ensure there is enough liquid in the wells.
	High pressure when PEEK tubing connected to probe; Normal pressure when tubing disconnected from probe.	Clogged stainless steel capillary in ion spray probe. Replace capillary.
	High pressure when PEEK tubing connected to probe, High pressure when tubing disconnected from probe.	Clogged PEEK tubing, injector valve, inline filter frit, or sample loop. Unclog or replace parts.
	Leak at finger-tight nut connecting PEEK tubing to ion spray probe.	Clogged stainless steel capillary in ion spray probe. Cracked PEEK tubing. Replace capillary. Replace PEEK tubing.
	Leak at injector-PEEK tubing connection.	Clogged stainless steel capillary in ion spray probe. Clogged PEEK tubing. Replace capillary. Replace PEEK tubing.
LC pump	No response.	The power is not turned on. Check electrical connections.
	No flow of the solvent when pump is run or purged.	Pump tubing is cracked, clogged, or leaking. Unclog/replace tubing. Flow solvent level low. Add additional flow solvent. Call service, check valves may need to be replaced.
	Pump leaking.	Loose valves. Defective check valves. Call service.

Equipment	Problem	Solutions
MS/MS (Quattro Micro)	Loss of signal.	Damaged cone, damaged stainless steel capillary, damaged probe tip. Replace parts. Reset the probe housing and make sure all electronic connections are tight. Reboot the system.
	Hardware communication problems.	Restart PC.
	Vacuum above/below acceptable range.	Check argon gas gauges. Call Proxy for advice on using the fine adjustment knob on MS/MS to adjust vacuum.
	Low intensity.	Clean cones. Replace capillary. Replace PEEK tubings. Ensure vacuum pressure is within acceptable range.
	Tail of chromatograph peak.	Probe tip needs to be changed. Adjust/replace capillary. Loose or leaking valves on LC pump.
	Chromatograph peak shift.	Air bubbles in tubing or capillary. Ensure PEEK tubing length is 5 feet. Change PEEK tubings. Change capillary.
	Unacceptable TIC shape (portions missing, “bat ears”, jagged top…).	Replace PEEK tubings. Replace capillary. Replace inline filter frit. Check LC pump pressure during priming to check that the pressure is within acceptable range. Check injector needle seal for clog. Ensure there is sufficient solvent in each well. Change syringe plunger. Sonicate the sample loop. Make sure gases and instruments are turned on. Change sample cone and gas cone nozzle.

C. Proxy Call Info

PerkinElmer™ life sciences.

Call Proxy (510) 215 - 1318

PerkinElmer LAS
855 Marina Bay Pkwy
Ste 31
Richmond, CA 94804
Phone: 510-237-9405
Fax: 510-237-9492
http://las.perkinelmer.com

NAPS TELEPHONE INSTRUCTIONS

1. Follow the prompts, **Press 1** for California then enter your **Lab Number** from **Table 1.**

Lab Number	Site
11	Western Clinical Laboratory
12	Allied Laboratory
21	Fresno Community Hospital
31	Quest Diagnostics
32	Memorial Med Center of Long Beach
62	Genetic Disease Lab – Laboratory
65	Genetic Disease Branch
69	Genetic Disease Lab- QA Room
71	Kaiser Permanente North
72	Kaiser Permanente South

Table 1: Lab Number

2. Wait until prompted then **Enter the System ID** from Listing on page 2.

*** See Page 2 for Complete 3 Digit System ID Listings ***

3. At prompt **enter a call back phone # ,** follow it by pressing the pound key (#).

4. After the Beep, **leave a voice message** give your name and a description of the problem. Press pound key (#) to end you voice message.

IMPORTANT → ***** Before hanging up *****

5. **Wait to hear this confirmation**, "A PerkinElmer Engineer will return your call".
6. **Done**

Listing - Enter the 3 digit ID					
ID	SYS	Description	ID	SYS	Description
151	AD1 P	AD1 PNS AutoDELFIA Plate Processor & PC	501	MM1 Q	MM1 Quattro Micro Mass Spec
152	AD2 P	AD2 PNS AutoDELFIA Plate Processor & PC	502	MM2 Q	MM2 Quattro Micro Mass Spec
153	AD3 P	AD3 NBS AutoDELFIA Plate Processor & PC	503	MM3 Q	MM3 Quattro Micro Mass Spec
154	AD4 P	AD4 NBS AutoDELFIA Plate Processor & PC	511	MM1 S	MM1 2777 AutoSampler
155	AD5 P	AD5 PNS AutoDELFIA Plate Processor & PC	512	MM2 S	MM2 2777 AutoSampler
156	AD6 P	AD6 PNS AutoDELFIA Plate Processor & PC	513	MM3 S	MM3 2777 AutoSampler
157	AD7 P	AD7 NBS AutoDELFIA Plate Processor & PC	521	MM1 P	MM1 Waters HPLC Pump
171	AD1 S	AD1 PNS AutoDELFIA Sample Processor	522	MM2 P	MM2 Waters HPLC Pump
172	AD2 S	AD2 PNS AutoDELFIA Sample Processor	523	MM3 P	MM3 Waters HPLC Pump
173	AD3 S	AD3 NBS AutoDELFIA Sample Processor	531	MM1 PC	MM1 Waters PC
174	AD4 S	AD4 NBS AutoDELFIA Sample Processor	532	MM2 PC	MM2 Waters PC
175	AD5 S	AD5 PNS AutoDELFIA Sample Processor	533	MM3 PC	MM3 Waters PC
176	AD6 S	AD6 PNS AutoDELFIA Sample Processor	561	PK1	MM1 PEAK N2 Generator & Argon Gas
177	AD7 S	AD7 NBS AutoDELFIA Sample Processor	562	PK2	MM2 PEAK N2 Generator & Argon Gas
201	CH1	Chrontrol Timer or Deep Well Plate Shakers	563	PK3	MM3 PEAK N2 Generator - Argon Gas
202	CH2	Chrontrol Timer or Deep Well Plate Shakers	611	HB40	HEATBLOCK 40 Degree VWR
211	TOM1	Tomtec 96 Well Pipettor	612	HB60	HEATBLOCK 60 Degree VWR
212	TOM2	Tomtec 96 Well Pipettor	621	PP1	Apricot Personal Pipettor
221	NG1	DBS Puncher for TSH, MSMS	622	PP2	Apricot Personal Pipettor
222	NG2	DBS Puncher for Universal Eluent - Hb	631	PP1 PC	Apricot PC
223	NG3	DBS Puncher for TSH, MSMS	632	PP2 PC	Apricot PC
224	NG4	DBS Puncher for Universal Eluent - Hb	641	SVR	Server PC
225	NG5	DBS Puncher for TSH, MSMS	651	TX1	3 Plate Thermomix INC/SHAKE
226	NG6	DBS Puncher for Universal Eluent - Hb	652	TX2	3 Plate Thermomix INC/SHAKE
241	BC1	PNS Barcode Printer	653	TX3	3 Plate Thermomix INC/SHAKE
242	BC2	NBS Barcode Printer	661	INC1	9 Plate Incubator Shaker
243	BC3	PNS Barcode Printer	662	INC2	9 Plate Incubator Shaker
244	BC4	NBS Barcode Printer	663	INC3	9 Plate Incubator Shaker
251	WAP1	PerkinElmer Wallac AutoPuncher	671	HS1	Heat Sealer MSMS
252	WAP2	PerkinElmer Wallac AutoPuncher	691	BR1	Branson Model 2510 Sonicator
301	SG1	Specimen Gate, Server, Router, File Transfer	692	BR2	Branson Model 2510 Sonicator
302	SG2	Specimen Gate, Server, Router, File Transfer	701	UT1	UT1 Transferase/Biotinidase System
303	SG3	Specimen Gate, Server, Router, File Transfer	702	UT2	UT2 Transferase/Biotinidase System
304	SG4	Specimen Gate, Server, Router, File Transfer	703	UT3	UT3 Transferase/Biotinidase System
311	RPC1	Specimen Gate Review Station PC	704	UT4	UT4 Transferase/Biotinidase System
312	RPC2	Specimen Gate Review Station PC	901	SS1	SIS- Data Terminal,Server or Scanner-PC
313	RPC3	Specimen Gate Review Station PC	902	SS2	SIS- Data Terminal,Server or Scanner-PC
314	RPC4	Specimen Gate Review Station PC	903	SS3	SIS- Data Terminal,Server or Scanner-PC
			904	SS4	SIS- Data Terminal,Server or Scanner-PC
			905	SS5	SIS- Data Terminal,Server or Scanner-PC
			906	SS6	SIS- Data Terminal,Server or Scanner-PC
			907	SS7	SIS- Data Terminal,Server or Scanner-PC
			908	SS8	SIS- Data Terminal,Server or Scanner-PC
			952	SC2	SIS - Optical Scanner
			951	SC1	SIS - Optical Scanner

XIII. MS/MS Plate Map

Full Tray

1	2	3	4	5	6	7	8	9	10	11	12
IS1	IS2	IS3	CL	CH	CT	P1	P2	P3	P4	P5	P6
P7	P8	P9	P10	P11	P12	P13	P14	P15	P16	P17	P18
P19	P20	P21	P22	P23	P24	P25	P26	P27	P28	P29	P30
IS	P31	P32	P33	P34	P35	P36	P37	P38	P39	P40	P41
CP	P42	P43	P44	P45	P46	P47	P48	P49	P50	P51	P52
P53	P54	P55	P56	P57	P58	P59	P60	P61	P62	P63	P64
P65	P66	P67	P68	P69	P70	P71	P72	P73	P74	P75	P76
P77	P78	P79	P80	P81	P82	P83	P84	P85	CT	CL	CH

Partial Tray

1	2	3	4	5	6	7	8	9	10	11	12
IS1	IS2	IS3	CL	CH	CT	PI	P2	P3	P4	P5	P6
P7	P8	P9	P10	P11	P12	P13	P14	P15	P16	P17	P18
P19	P20	P21	P22	P23	P24	P25	P26	P27	P28	P29	P30
IS	P31	P32	P33	P34	P35	P36	CT	CL	CH		

1. *IS = Internal Standard*
2. *CL = Low System Control*
3. *CH = High System Control*
4. *CT = Tray Control*
5. *CP = Proficiency Control (Same as Tray Control)*

MS/MS PLATE MAP

Kit Lot No._____

	1	2	3	4	5	6	7	8	9	10	11	12
A	IS1	IS2	IS3	CL	CH	CT	Patient					
B												
C												
D	IS											
E	CP											
F												
G												
H										CT	CL	CH

IS = Internal Standard
CL= Low System Control
CH= High System Control

CT= Tray Conrol
CP= Proficiency Control (Same as Tray Control)

XIV. MS/MS Maintenance/Preventive Logs

(Daily) GAS Pressure Log

MS/MS Unit: _____ **Month/Year**: **_____**

Date	Time	Analyst	N2 Generator pressure (psi) (90-120)	Argon outlet pressure @ tank (psi) (25-30)	Argon gauge pressure @ MS/MS (psi) (8-10)	Empty the Condensation bottle once a week	Comments

Comments: _____
_____
_____
_____

Temperature Log (Incubator/Shaker Unit)

Serial Number: __________________

Month/Year: __________________

Temperature 45°C (44-46°C) Place Number 1, 2, or 3 **Shaker @ 700 (650-750 rpm)**				
Date	Analyst	Place Number	Start Temp	End Temp

Supervisor: ____________ Date: ___________

Month/Year: __________________

Temperature 45°C (44-46°C) Place Number 1, 2, or 3 **Shaker @ 700 (650-750 rpm)**				
Date	Analyst	Place Number	Start Temp	End Temp

Supervisor: ____________ Date: ___________

Preventive Maintenance Log for Quattro micro (DAILY)

Supervisor:_____

MS/MS Unit:_____

Year: _____

Date	Analyst initials	Restart PC's	Check vacuum light on MSMS (green)	Check/ Refill Flow Solvent	Check/ Refill Wash Solvent	Sonicate cones with MeOH	Replace In-Line Filter every other day	Check injection port & Teflon tubing for clog; flush w/ MeOH	Clean/ Prime syringe 5 times	Prime LC pump	LC Pump psi during Prime	Check for leaks	Clean ionization chamber w/MeOH	Vacuum gas cell pirani pressure when Collision gas ON $(2.7\pm0.5)\ e^{-3}$

Comments:_____

_____

_____

Preventive Maintenance Log for Quattro micro (Weekly)

MS/MS Unit:_____ **Year:_____**

Date	Analyst	Sonicate sample cones in 10% Formic Acid/Methanol	Ballast vacuum pump For 15 minutes	Replace Syringe Unit as Needed	Supervisor

Comments:_____
_____

Preventive Maintenance Log for Quattro micro (Monthly)

MS/MS Unit:_____ **Year:_____**

Date	Analyst	Change red & yellow PEEK tubing	Check oil level/color in vacuum pump	Supervisor

Comments:

_____
_____
_____
_____
_____

Preventive Maintenance Log for Apricot

Year:_____ **Month:_____**		**Daily** **MS/MS Unit:_____**				**Monthly**	
Date	**Analyst**	**Change wash water (DI water)**	**Oil Apricot tip head**	**Change Apricot tips**	**Remove tip head after last use of the day**	**Add oil to lubrication box**	**Supervisor**

Comments:_____

Preventive Maintenance Log for Incubator/Shaker (Weekly)

MS/MS Unit:_____ Serial Number: _____ Year:_____

Date	Analyst	Wipe the outside, inside and each plate holders with a damp cloth.	Supervisor

Comments: _____

_____

_____

Preventive Maintenance Log for DBS Puncher

Year:_____		Weekly	(Monthly) MS/MS Unit:_____			Supervisor
Date	Analyst	Remove & clean punch head with MeOH	Remove, clean, & store punch head	Install 2nd punch head	Clean puncher unit with moist cloth	

Comments:_____
_____
_____

Preventive Maintenance Log for Sonicator Bath (Monthly)

MS/MS Unit:_____ **Year:_____**

Date	Analyst	Empty & wipe inside of bath with moist Kimwipe	Refill sonicator with d.i. water and run for 15 mins.	Supervisor

Comments:

_____
_____
_____
_____
_____

MS/MS 100 -1000µl Single-channel Pipette

A&P Log for Volume at 100 µL (Quarterly)

Date: _____ Analyst: _____ Pipette ID: _____

Room Temp. = _____ °C = _____ (Temp. correction factor)

	Cup #	Tare Weight (g)	Final Weight (g)	Average Volume Dispensed (Final weight-tare weight) x 1000 µL / Temp. Correction Factor
Volume Dispensed	1			
	2			
	3			
	4			
	5			

Average Volume	
Standard Deviation	
CV=(SD/Ave)x100%	

A&P Log for Volume at 1000 µL (Quarterly)

Date: _____ Analyst: _____ Pipette ID: _____

	Cup #	Tare Weight (g)	Final Weight (g)	Average Volume Dispensed (Final weight-tare weight) x 1000 µL / Temp. Correction Factor
Volume Dispensed	1			
	2			
	3			
	4			
	5			

Average Volume	
Standard Deviation	
CV=(SD/Ave)x100%	

Supervisor: _____ Date: _____

MS/MS 20 - 200µL Single-channel Pipette

A&P Log for Volume at 100 µL (Quarterly)

Date: _____ Analyst: _____ Pipette ID: _____

Room Temp. = _____ °C = _____ (Temp. correction factor)

	Cup #	Tare Weight (g)	Final Weight (g)	Average Volume Dispensed (Final weight-tare weight) x 1000 µL / Temp. Correction Factor
Volume Dispensed	1			
	2			
	3			
	4			
	5			

Average Volume	
Standard Deviation	
CV=(SD/Ave)x100%	

Supervisor: _____ Date: _____

MS/MS Multi-Channel Pipette Accuracy Log for Volume at 100 µL (Quarterly)

Date: _____ Analyst: _____ Pipette ID: _____

Room Temp. = _____ °C = _____ (Temp. correction factor)

	Cup #	Tare Weight (g)	Final Weight (g)	Average Volume Dispensed (Final weight-tare weight)/96 x 1000 µL / Temp. correction factor
Volume Dispensed	1			
	2			
	3			
	4			
	5			

Average Volume	
Standard Deviation	
CV=(SD/Ave)x100%	

Precision Log for Volume at 100µL (Quarterly)

Date: _____ Analyst: _____ Pipette ID: _____

Well-to-Well Precision	
Plate CV for 100 µL	Plate CV Limit for 100 µL
	5%

Supervisor: _____ Date: _____

MS/MS Multi-Channel Pipette Accuracy Log for Volume at 75 µL (Quarterly)

Date: _____ Analyst: _____ Pipette ID: _____

Room Temp. = _____ °C = _____ (Temp. correction factor)

	Cup #	Tare Weight (g)	Final Weight (g)	Average Volume Dispensed (Final weight-tare weight)/96 x 1000 µL / Temp. correction factor
Volume Dispensed	1			
	2			
	3			
	4			
	5			

Average Volume	
Standard Deviation	
CV=(SD/Ave)x100%	

Precision Log for Volume at 75µL (Quarterly)

Date: _____ Analyst: _____ Pipette ID: _____

Well-to-Well Precision	
Plate CV for 100 µL	Plate CV Limit for 100 µL
	5%

Supervisor: _____ Date: _____

Appendix 5F

Apricot Accuracy and Precision Record (Monthly)

Date			Analyst
	Well-to-well Precision		
	Plate CV Limit for 75 µL	**Plate CV for 75 µL**	
	5%		
	Volume Accuracy		
	Volume Tested	**75 µL**	
	Volume limit range	**± 5% (72.75 µL -77.25 µL)**	
	Actual average volume delivered to each well		

Room Temp. = _____ °C = _____ (Temp. correction factor)

Supervisor: _____ Date: _____

XV. Checklist

MS/MS CHECKLIST (AA/AC)

Laboratory Site: _____ *PE Kit Lot No.* _____

Test Date	MS ID	Slot No.	Tray ID (last 4 digits or tray barcode)	Accession Date (*Julian*)	*For NAPS Laboratory*	*For GDLB*	
					Releaser Name & Date & Time	Reviewer Name & Date	Releaser Name & Date

For NAPS Laboratory Use Only

Are any Specimens delayed more than 2 days? Yes _____ No _____
Are any Instruments down today? Yes _____ No _____
If Yes, please describe:
_____
_____

Repeats from Your Lab? Yes_____ No_____

Data Transferred? Yes_____ Initial _____

Date: _____ Time:_____ AM/PM

Comments:
_____
_____

Comments:

XVI. Limitations of the Procedure

A. The NeoBase Non-derivatized MSMS kit is a screening assay, not a diagnostic test. As with any other in vitro screening test, the data obtained using this kit should be used as an aid to other medically established procedures and results interpreted in conjunction with other clinical data available to the clinician. A diagnostic procedure should be used for confirmation of presumptive abnormal amino acid, succinylacetone, free carnitine and acylcarnitine profiles.

B. Conditions which are known to cause anomalous assay results are
- Sample spot not uniformly saturated with blood
- Sample punched too close to the edge of the blood spot
- Sample collected and improperly dried
- Non-eluting blood spot
- Contamination of blood spot

C. Hydroxyproline can contribute to the Leu + Ileu marker signal. Hydroxyproline is present in low concentrations in the newborn and its signal is less than 1% of the corresponding amount of the Leu + Ileu signal.

D. Antibiotics that contain pivalic acid (pivoxilsublactam) can be metabolized to pivaloylcarnitine, which can cause false positive results for Isovaleric Acidemia (C5).

E. Total Parental Nutrition (TPN) will cause an increase in the levels of essential amino acids (such as Phenylalanine and Leucine), possibly free carnitine and other acylcarnitines (such as C5 and C8). Many premature babies may be on special formula

Appendix A
Sample Cone Cleaning Procedure (from Perkin Elmer)

A. Preparation of the Sonicator with beaker

DO NOT PUT CLEANING SOLUTIONS OR PARTS DIRECTLY INTO SONICATOR

1. Place the beaker cover on the Branson 2510 sonicator.
2. Add 100-150 mL of methanol and water (50:50) cleaning solution to a 600 mL beaker.
3. Place the beaker with cleaning solution into one side of the beaker cover.
4. Fill the tank with cleaning distilled water to within 3/8 inch of the operating level line marked on the inside of the sonicator tank. Do not overfill.
5. Gently lower the sample cone and gas nozzle into the beaker. Be sure the parts are completely covered with methanol and water (50:50) cleaning solution.
6. Plug Sonicator into 115v AC outlet.

B. Storage

1. Unplug the sonicator from the AC power source.
2. Remove the beaker and properly dispose cleaning solution.
3. Cover the tank when not in use.
4. Check the Sonicator tank water weekly and if cloudy or dirty change it before next use.
5. If it is necessary to empty the tank, unplug the Sonicator from the AC power source. Pour the water out from the indented side of the rim.

C. Daily Sample Cone Cleaning

1. Cover sample cone and gas nozzle (out cone) with approximately 150 mL methanol and water (50:50) in the 600 mL cleaning beaker. Do not sonicate the cone **O-Ring**.
2. Sonicate for 15 minutes. Remove from beaker and inspect for cleanliness. Repeat one or two times if necessary.
3. When the cone appears to be clean, allow excess methanol to evaporate and re-install for normal use. The outer cone may be gently wiped with a Kimwipe or lint free cloth. Do not wipe or rub the inner sample cone.

D. Weekly Cone Cleaning:

1. Weekly cleaning is the same as daily cleaning procedure with the exception of adding formic acid to the cleaning solution but only in the event of stubborn burn marks or discoloration. Follow normal daily cleaning procedure.
2. Remove from beaker and inspect the sample cone and gas nozzle for cleanliness. If burn marks, discoloration, etc. are observed, go to step 5.
3. If the cone appears to be clean, allow excess methanol to evaporate and re-install for normal use.
4. Add approximately 15 mL formic acid to 150 mL methanol to obtain a 10 % Formic Acid cleaning solution.
5. Sonicate for 15 minutes per section, repeat if necessary. Contact PerkinElmer if the cone remains unclean after performing the cleaning procedure.

Appendix B

Conversion of mass of water to volume of water at selected temperatures
(Temperature Correction Factor)

	Mass	**Volume**
°C	**g/cm^3 ***	**g/mL ****
18.0	0.9985986	**0.9986266**
18.5	0.9985034	**0.9985314**
19.0	0.9984082	**0.9984362**
19.5	0.9983077	**0.9983356**
20.0	0.9982071	**0.9982350**
20.5	0.9981013	**0.9981292**
21.0	0.9979955	**0.9980234**
21.5	0.9978845	**0.9979124**
22.0	0.9977735	**0.9978014**
22.5	0.9976575	**0.9976854**
23.0	0.9975415	**0.9975694**
23.5	0.9974205	**0.9974484**
24.0	0.9972995	**0.9973274**
24.5	0.9971737	**0.9972016**
25.0	0.9970479	**0.9970758**
25.5	0.9969173	**0.9969452**
26.0	0.9967867	**0.9968146**
26.5	0.9966515	**0.9966794**
27.0	0.9965162	**0.9965441**
27.5	0.9963764	**0.9964042**
28.0	0.9962365	**0.9962644**

* density of water (CRC Handbook of Chemistry and Physics)

** conversion of cm^3 to mL (CRC Standard Math Tables, 1.000028 cm^3/mL)

XVII. References

1. Bhandal A, Ho T, Onuska K, Sherwin JE. Comparison of Derivatized and Non-Derivatized Tandem Mass Spectrometry Methods for the Detection of Metabolic Disorders in Newborns. Paper presented at the 2004 Newborn Screening and Genetic Testing Symposium in Atlanta, GA. (May 5, 2004)

2. Bhandal A, Sherwin JE, Cunningham GC, Levine S, Currier B. California Tandem MS Research Project Jan 2002-July 2003 Final Results. Paper presented at the 2004 Newborn Screening and Genetic Testing Symposium in Atlanta, GA. (May 3-6, 2004)

3. Bhandal A, Sherwin JE, Robello M, Nikbakht M, Campbell T, Currier B, Lorey F, Cunningham G. California Expanded Newborn Screening Program with Tandem Mass Spectrometry: Establishing common cutoffs for use on multiple MS/MS instruments at multiple screening sites. Paper presented at the 2005 Newborn Screening and Genetic Testing Symposium in Portland, OR. (October 27, 2005)

4. Chace DH, Millington DS, Terada N, Kahler SG, Roe CR, Hofman LF. Rapid Diagnosis of Phenylketonuria by Quantitative Analysis for Phenylalanine and Tyrosine in Neonatal Blood Spots by Tandem Mass Spectrometry. Clin Chem 39, 66-71 (1993)

5. Chace DH, Hillman SL, Van Hove JLK, Naylor EW. Rapid diagnosis of MCAD deficiency: quantitative analysis of octanoylcarnitine and other acylcarnitines in newborn blood spots by tandem mass spectrometry. Clin Chem 43.2106-2113 (1997)

6. Chace DH, Sherwin JE, Hillman SL, Lorey F, Cunningham GC. Use of phenylalanine-to-tyrosine ratio by tandem mass spectrometry to improve newborn screening for phenylketonuria of early discharge specimens collected in the first 24 hours. Clin Chem 44.2405-2409 (1998)

7. PerkinElmer Life and Analytical Sciences, Wallac Oy, Mustionkatu 6, Turku, Finland, NeoBase Non-derivatized MSMS Kit Instruction for use, Document ID 3030-0011U

Prepared by**:** _____ Date: _____

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Reviewed by: _____ Date: _____

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Reviewed by: _____ Date: _____

CLIA Laboratory Director, MD

Approved by: _____ Date: _____

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Procedure Revision Log

Enter section(s) and page number(s) where deletion, revision or add-on is done and indicate it with an "X" in the appropriate column.

Procedure: Tandem Mass Spectrometry Method for the Analysis of Amino Acids, Acylcarnitines, and Succinylacetone, Tracking No CN 009, Version 2.0

Section	Page #	Deletion	Revision	Add-on
IV.B.h.	4		X	
V.A.1.a.NOTE	5		X	
VII.C.1.b and f	7		X	
VII.C.2.a	7		X	
VII.D.19.Note	Was 10	X		
VII.E.2,9,14, 15	10-12		X	
VII.G	12-15		X	
VII.H.3	16		X	
VII.J.1.b.8 moved to VII.J.1.c.12	Was 19 back to 18		X	
VII.J.1.c.11	18			X
VII.K.1.d	19			X
VII.L.2.c.Note	20		X	
VII.N	20-27		X	
VII.N.6.c, Biweekly	24	X		
IX. C. NOTE	28			X
VII.N.6.c.3), Ver. 1.2	Was 32	X		
Table 1, Masses of Biochemical Markers	30 - 31		X	
Table 2, Cutoff for AA, SUAC, AC	32 - 33		X	
Table 4, Disorder Detected	35 - 36		X	
Temp. Log	46		X	
Preventive Maintenance Log for QM (weekly)	48		X	
A&P Logs for pipettes	54-57		X	X
Auto Sampler Syringe Plunger Ver. 1.4	Was 60	X		
A&P Log, 100-1000µL Single-channel pipette, 100 and 800 µL	Was 61	X		

Procedure Revision Logs (continued)

Section	Page #	Deletion	Revision	Add-on
A&P Log, 20 – 200 µL Single-channel pipette, 100 µL	Was 62	X		
A&P Log, 50 – 300 µL Multi-channel pipette, 100 µL	Was 63	X		
A&P Log, Multi-channel pipette at 75 µL, Ver 1.2	Was 64	X		
Appendix B	62			X
New Version	All			

Technical Performance Verification

Procedure: Tandem Mass Spectrometry Method for the Analysis of Amino Acids, Acylcarnitines, and Succinylacetone, Tracking No CN 009, Version 2.0

Employee's Signature[1]	Supervisor's Signature[2]	Date of Completion

1. Employee's Signature signifies that the employee is confident in performing the procedure.

2. Supervisor's Signature signifies that department supervisor is satisfied with employee's competence to perform the procedure.

Procedure Review and Update Log

All procedures should be reviewed every 12 months. The Laboratory Director must also approve all new methods and procedural changes.

Procedure: Tandem Mass Spectrometry Method for the Analysis of Amino Acids, Acylcarnitines, and Succinylacetone, Tracking No CN 009, Version 2.0

Date	Supervisor's signature	Procedure Version	Review	Update	New Method	Laboratory Director's signature	Date